AWARD NUMBER: W81XWH-14-1-0067

TITLE: Testing Current and Developing Novel Therapies for NF1-Mutant Sarcomas in a Genetically Engineered Mouse Model

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REPORT DATE: April 2015

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
April 2015	Annual	15 Mar 2014 - 14 Mar 2015

4. TITLE AND SUBTITLE

Testing Current and Developing Novel Therapies for NF1-Mutant Sarcomas in a Genetically Engineered Mouse Model

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-1-0067

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Duke University
Durham, NC 27708

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Patients with Neurofibromatosis type 1 (NF1) are at increased risk for developing malignant tumors of the connective tissue called soft-tissue sarcomas, including malignant peripheral nerve sheath tumor (MPNST), rhabdomyosarcoma (RMS), and undifferentiated pleomorphic sarcoma (UPS). These tumors are highly aggressive, with 5-year survival rates of 50-60%. We have developed novel mouse models of temporally and spatially restricted soft-tissue sarcoma in $NF1^{flox/flox}$; $Ink4a/Arf^{flox/flox}$ mice. Following injection of an adenovirus expressing Cre recombinase (Ad-Cre), these mice develop MPNSTs after injection into the sciatic nerve or high-grade myogenic sarcomas (RMS/UPS) after injection into the muscle. During this research period, we have also developed an improved model that accelerates tumor formation while preserving the fidelity of this model. We are using these models as preclinical platforms to investigate the impact of the tumor microenvironment on sarcoma response to chemotherapy and to test novel approaches for treating NF1-mutant sarcomas. These studies may identify more efficacious treatments for patients with NF1-mutant sarcomas.

15. SUBJECT TERMS
sarcoma, mouse model, MPNST, Neurofibromatosis, NF1

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	9	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1. INTRODUCTION:

Patients with Neurofibromatosis type 1 (NF1) are at increased risk for developing malignant tumors of the connective tissue called soft-tissue sarcomas, including malignant peripheral nerve sheath tumor (MPNST), rhabdomyosarcoma (RMS), and undifferentiated pleomorphic sarcoma (UPS). These tumors are highly aggressive, with 5-year survival rates of 50-60%. Despite the prevalence of sarcomas in NF1 patients, there is an unmet need to further explore the role of NF1 in soft-tissue sarcomas and to develop new treatment modalities. We are using novel mouse models to investigate the impact of the tumor microenvironment on sarcoma response to chemotherapy and to test novel approaches for treating NF1-mutant sarcomas. To model the unique genetics of the NF1-associated tumor stroma, we have developed novel mouse models of temporally and spatially restricted soft-tissue sarcoma in $NF1^{flox/-}$; Ink4a/$Arf^{flox/flox}$ and $NF1^{flox/flox}$; Ink4a/$Arf^{flox/flox}$ mice. Following injection of an adenovirus expressing Cre recombinase (Ad-Cre), these mice develop MPNSTs after injection into the sciatic nerve or high-grade myogenic sarcomas (RMS/UPS) after injection into the muscle. These tumors faithfully reflect the spectrum of sarcomas found in NF1 patients. We will use our NF1-deleted sarcoma mouse models to define the role of NF1 haploinsufficient stroma in the therapeutic response to standard-of-care chemotherapies. In addition, we will test novel formulations of chemotherapy and a unique radiation sensitizer. By clarifying the role of the tumor stroma in chemotherapy response, our results will inform the design of future clinical trials for neurofibromatosis patients with MPNSTs. Our preclinical studies with novel chemotherapy and radiosensitizers may identify more efficacious treatments for NF1-mutant sarcomas. If successful, these data will be used to support phase I clinical trials for patients with NF1-mutated soft-tissue sarcoma.

2. KEYWORDS:

NF1, neurofibromatosis, sarcoma, MPNST, mouse model, preclinical study, tumor microenvironment, molecularly-targeted therapy, chemotherapy, radiation oncology,

3. ACCOMPLISHMENTS:
What were the major goals of the project?

There are three major goals for the project during this time period stated in the approved SOW.

The first major goal was to determine the influence of NF1 haploinsufficient stroma on chemotherapeutic response using a primary tumor model. Three subtasks were scheduled for this time period in the SOW. The first subtask was to go through regulatory review and the approval processes from IACUC and ACURO for animal use (months 1-3). This approval was obtained from USAMRMC on March 24, 2014. The second subtask was to breed the paired littermate mice necessary for study (months 3-8). During this time, we have generated a large cohort of parental breeders and

experimental mice for the upcoming studies. The third subtask was to generate MPNSTs using Ad-Cre injection in the mice generated in subtask #2 (months 6-12). We have successfully injected the mice in this cohort.

The second major goal was to examine the efficacy of nanoparticle-encapsulated doxorubicin in mouse models of NF1-deleted sarcoma. One subtask was scheduled for this time period in the SOW. This subtask was to breed the mice necessary for study (months 6-12). We have successfully bred the mice for the upcoming study.

The third major goal was to examine efficacy of the radiosensitizer BEZ325 in mouse models of NF1-deleted sarcoma. One subtask was scheduled for this time period in the SOW. This subtask was to breed the mice necessary for study (months 6-12). We have successfully bred the mice for the upcoming study.

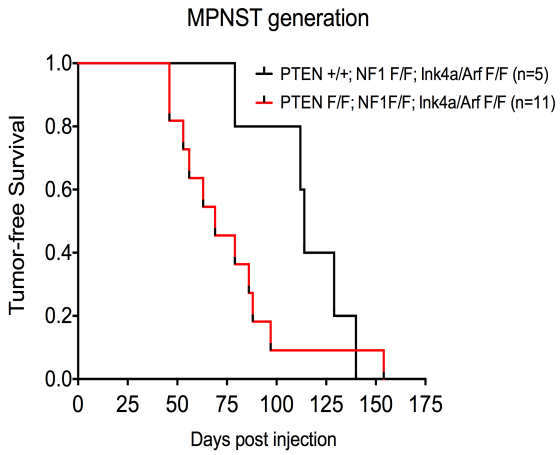
What was accomplished under these goals?

The major activity for this reporting period was to obtain regulatory approval and generate a large cohort of mice for the upcoming studies. These objectives have been met as outlined above.

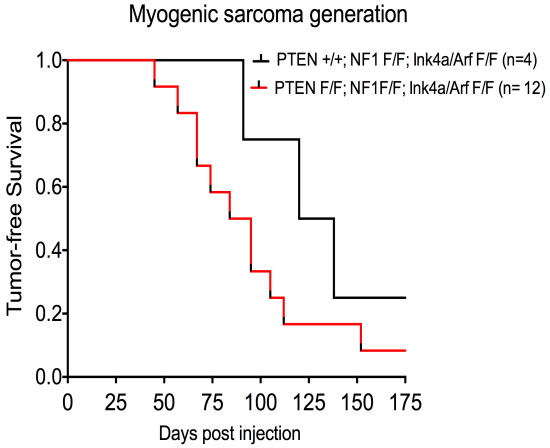
During this time, we reasoned that the project would benefit from an accelerated tumor model. The current model requires 4 months to generate MPNSTs and 6-8 months to generate myogenic sarcoma (RMS/UPS). Accelerating tumor development would greatly facilitate the proposed experiments for preclinical studies. Thus, a major activity for this period was to develop an improved model for tumor generation. We have successfully developed and characterized an additional model that drastically reduces the time required for tumor development. This new model incorporates deletion of the PTEN allele, a tumor suppressor involved in NF1-related sarcoma development. We directly compared this modified model ($PTEN^{flox/flox}$; $NF1^{flox/flox}$; Ink4a/$Arf^{flox/flox}$ mice) to our original model ($PTEN^{+/+}$; $NF1^{flox/flox}$; Ink4a/$Arf^{flox/flox}$ mice). Deletion of two alleles of PTEN accelerated tumor formation from an average of 4 months to 2.5 months (MPNSTs) and from 6 months to 2.6 months (myogenic tumors) (Figure 1A and B). We extensively characterized these tumors to assure they are phenotypically similar to the original model and faithfully reflect the spectrum of sarcomas found in NF1 patients. We performed extensive immunohistochemical analysis of the two models and find similar staining of the sarcoma markers MyoD, S100, and myogenin, in addition to similar immune cell populations. Loss of PTEN protein expression was verified by immunohistochemistry. Additionally, growth rates of PTEN-deleted tumors were similar to PTEN wild-type tumors. Thus, we believe this model is an important development in our studies that will generate tumors in a shorter time period.

Figure 1. Impact of PTEN loss on tumor generation. (A) MPNST generation following Ad-Cre injection is accelerated with PTEN loss. (B) Myogenic sarcoma generation following Ad-Cre injection is accelerated with PTEN loss.

A.



B.



What opportunities for training and professional development has the project provided?

This project has provided scientific training for two high school students. Mr. Matt Mosca, a high-school sophomore, worked in our lab over the summer and received scientific training analyzing tumor tissues related to this project. Ms. Tess Overton, a high-school senior, participated in an 8-month scientific mentorship program that focused on aspects of this project.

A senior post-doc in the lab, Dr. Rebecca Dodd, attended the Children's Tumor Foundation meeting to discuss work related to this project.

How were the results disseminated to communities of interest?

Data related to this project were presented at the Children's Tumor Foundation (CTF) in June 2014. The CTF is the largest non-governmental organization focusing on NF1-releated research. This meeting is an outstanding platform for disseminating results to the NF1 research community of interest.

What do you plan to do during the next reporting period to accomplish the goals?

During the next reporting period, we anticipate initiating and completing the preclinical study examining the influence of NF1 haploinsufficient stroma on chemotherapeutic response in our primary tumor model. We will treat MPNSTs with either doxorubicin+ifosfamide or vehicle alone and follow tumor growth. If these treatment studies are successful, we anticipate performing histopathological and

immunohistochemical studies on tissues from treated MPNSTs. We also plan to generate cell lines from mouse MPNSTs for use in future studies.

In addition, we will generate tumors for upcoming preclinical studies. Once these tumors are detected, we will begin assessing novel chemotherapy formulations by treating MPNSTs and myogenic sarcomas with either nanoparticle-encapsulated doxorubicin or vehicle alone. We will also begin assessing a novel radiosensitizer by treating MPNSTs and myogenic sarcomas with either radiation alone or radiation plus the radiosensitizer BEZ235. These treatment studies will extend past the next reporting period and into the third year of funding.

IMPACT:
What was the impact on the development of the principal discipline(s) of the project?

The development of a more rapid tumor model will positively impact the ability to perform preclinical studies in mouse models of NF1-related sarcoma. This will reduce overall costs and accelerate discovery studies.

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

CHANGES/PROBLEMS:
Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals.

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

PRODUCTS:
Publications, conference papers, and presentations

Conference Paper:
Presented at Children's Tumor Foundation Meeting, June 2014:

Title: NF1 deletion generates multiple subtypes of soft-tissue sarcoma that respond to MEK inhibition

Authors: Rebecca Dodd, Jeffrey Mito, Will Eward, Mohit Sachdeva, Rhea Chitalia, Yan Ma, Leslie Dodd, & David Kirsch

Journal publications.

Nothing to Report

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers, and presentations. *Identify any other*

Nothing to Report

Website(s) or other Internet site(s)

Nothing to Report

Technologies or techniques

Nothing to Report

Inventions, patent applications, and/or licenses

Nothing to Report

Other Products

Nothing to Report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS
What individuals have worked on the project?

David Kirsch, MD, PhD – no change
Rebecca Dodd, PhD – no change
Yan Ma, BS – no change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

Nothing to Report

SPECIAL REPORTING REQUIREMENTS Nothing to Report

COLLABORATIVE AWARDS: None
QUAD CHARTS: None
APPENDICES: None